PINPOINT INJECTION OF MICRO TOOLS USING DIELECTROPHORESIS AND HYDROPHOBIC SURFACE FOR MINIMALLY INVASIVE SEPARATION OF MICROBE

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ABSTRACT

We proposed a pinpoint injection method of micro tools (MTs) at the desired location in the micro chamber, which is filled with liquid. We demonstrated a new method to install the micro tools inside the micro chamber. We developed a micro tool holding chip to install the micro tools. The micro tools were aligned in the micro groove autonomously by the dielectrophoresis and liquid bridge force of the hydrophobic surface. Then, MTs were injected in the micro chamber, and were manipulated successfully by the laser scanning micromanipulator to separate the target microbe. It is the first demonstration of the pinpoint injection of MTs and separation by the indirect micromanipulation.

INTRODUCTION

Recently, there has been great interest in the high throughput screening of microorganisms, for example, for finding of novel microbes. It is estimated less than 10% within the whole in which the microorganisms known at present passed. The size of most microbes is around 1-10 μm(bacteria: 1-3μm, yeast: 4-10μm). It is important to develop a new technology, with which we can manipulate and separate randomly suspended microorganisms with high speed and high purity. The flow cytometer (cell sorter) is well known as a selection system for the plant cell (size: around 20 μm)[1]. However, the separation process is sequential and it is not suitable for random selection & separation of the single target.

We have studied on the selective separation of the microbe suspended in the liquid. In the previous works[2]-[4], we integrated the laser tele-manipulator for local position control of the target, and the dielectrophoresis for exclusion of the other objects around the target by shaping the potential field in the microchannel. An arbitrary single microbe was isolated speedily in a microchannel, even though there are a large number of microbes in solution Then the target microbe was transported along the proper transportation trajectory. It was released around the split of the microchannel flow. Then it was taken out by the high speed flow in the microchannel. To reduce the separation time, we shortened the transportation distance of the laser. We developed a separation cell having modified microchannel system with stagnation point control by the pressure adjustment. Pressure balance was adjusted properly and the stagnation point in the microchannel was controlled to realize high speed and high purity extraction of the target [4].

The laser trapping method has a problem of direct irradiation of high power laser to the target. For safe and secure transportation of the microbe, we proposed to transport a microbe with micro tools trapped by the laser (indirect laser micromanipulation). The indirect micromanipulation of the microbe was reported by us at MEMS'00[2]. To realize the indirect manipulation of the microbe, we have to consider how to inject such a small tool at the desired location. Injection method of the micro tool has not been reported before. Our method employs unique technique to fix the micro tool in the micro groove by the dielectrophoresis and liquid bridge force of the hydrophobic surface before immersion, and to release it automatically after immersion by employing the water-soluble layer. This is the first demonstration of the pinpoint injection of MTs and separation by the indirect micromanipulation.

LASER MANIPULATION SYSTEM

We built a laser scanning tele-manipulation system with bilateral feedback[5]. we installed two different lasers. One is the CW Nd:YVO_4 laser (wave length: 1064 nm, maximum power 4.98 W, mode: TEM_{00}, M^2<1.1) and the other is the semiconductor laser (wave length: 860 nm, maximum power 200 mW). Focus of each laser is adjusted independently, and is scanned by the Galcano mirrors in the observation plane. At this moment, The main body was made by the die casting to have the laser scanning micromanipulator and the inverted microscope. The separation cell is set on the XY stage of the inverted microscope. The XY stage is controlled by the stepping motors and PZT actuators. The operator can control both laser focal points by the force feedback joystick, and can

laser-trapped object by watchin it in the monitor.

INDIRECT MICROMANIPULATION

There have been reported that direct irradiation of the laser beam may give some damage to the trapped object[6]. This phenomenon depends on the target, wave length, and power of the laser. To evade this problem, we proposed the indirect manipulation of the target by the laser trapped micro tools[2]. As an example of a micro tool, we tested a Lactobacillus bulgaricus (LB), polystyrene beads as a micro chopsticks, and a liposome as a micro capsule[2]. The target was pushed by the laser trapped MT. Since the spot of the laser focus is reduced less than a few μm, there is no remarkable interference between the target and the tool. This means minimally invasiveness to the target in the indirect micromanipulation by the laser trapped micro tools. If we design the micro tool properly, transportation of the target will become easy. However, it is difficult to inject the proper number of micro tools at the working area. An excess of micro tools disturb separation woks. From this reason, here we propose pinpoint injection of the moderate number of micro tools in the separation area.

PINPOINT INJECTION OF MICO TOOLS

Classification of Injection Method

The size of the micro tool is around micrometer. The micro tools are manipulated by the focused lasers under the microscope to manipulate the target microbe. Here we propose a pinpoint injection method of micro tools at the desired location in the micro chamber, which is filled with liquid.

At first, we classified the injection method of the micro tools in 4 categories as shown in Fig. 1.

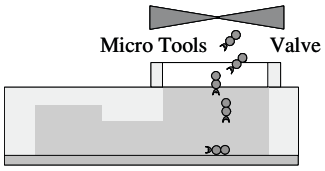
(a) Outer injection method:
The tools are injected outside.

(b) Inner assembly method:
The parts are assembled inside.

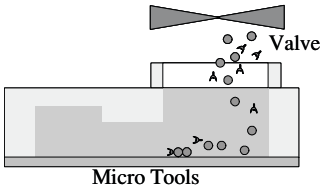
(c) Inner fabrication method
The tools are fabricated inside.

(d) Inner installation method
The tools are set inside and detached properly.

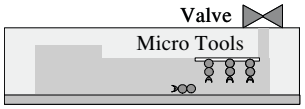
Here we employed a new method to install the micro tools inside the micro chamber, which we call “Inner installation method” as shown in Fig. 1 (d). Figure 2 shows the injection process of the micro tools. At first, the micro tools are set on a micro tool (MT) holding chip. The MT holding chip is set bottom up in the micro chamber and is filled with water. Then, the fixed MTs on the MT holding chip surface are released in the water, since the water-soluble layer is dissolved. In this way, the MTs are injected beneath as shown in Fig. 3, so it is easy to use it as the functional device to manipulate the target microbe in the micro chamber.



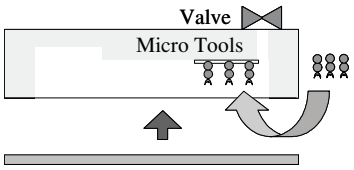
(a) Outer injection method (Side view)



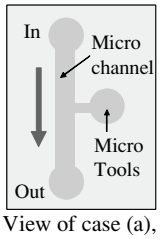
(b) Inner assembly method (Side view)



(c) Inner fabrication method (Side view)



(d) Inner installation method (Side view)



(e) Top View of case (a), (b), (c), (d)

Fig. 1 Injection methods of micro tools

Pinpoint Injection by Inner Installation

We developed a micro tool holding chip, which has two electrodes and a micro groove (Fig. 4). The surface of the micro groove is hydrophobic. A drip of diluted surfactant with dispersed MTs is put on the micro groove. Then we apply the AC electric field between the electrodes to control the position of the MTs. The dielectrophoretic force is generated on the MTs by the field gradient between two electrodes. If the permittivity of the MT is smaller than that of the solvent, the MT moves toward low electric field gradient

direction by the negative dielectrophoretic force. In case of negative dielectrophoresis, some MTs migrate toward the center of the micro groove or move away from the micro groove as shown in Fig. 5. The motion of the MT depends on its initial position. The MTs gathered in the micro groove are aligned along the hydrophobic surface autonomously by the liquid bridge force. Finally, the liquid evaporates and the MTs are fixed firmly on the bottom of the micro groove with the water-soluble layer.

Experiment of Pinpoint Injection and Manipulation

For demonstration, we fabricated a new separation cell having a micro channel and micro chamber. It is fabricated with the transparent silicone elastomer - polydimethylsiloxane (PDMS) using the replica molding technique[10]. Diameter of the separation cell is 76 mm. It is shown in Fig. 6.

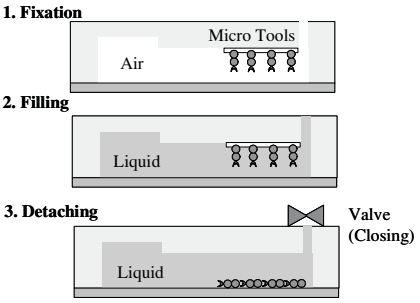


Fig.2 Inner Installation Method (Side View)

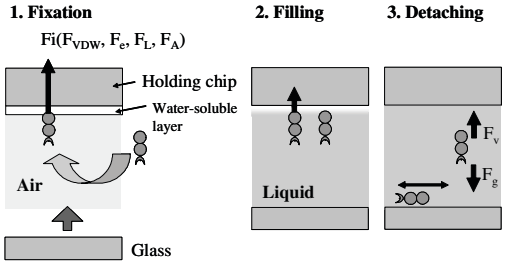
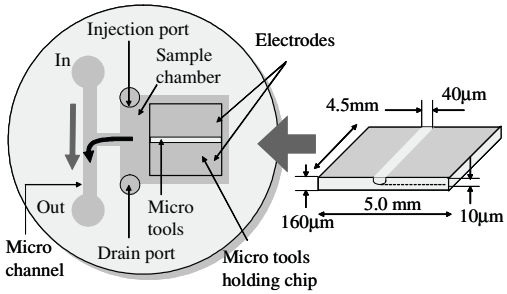


Fig. 3 Release of the Micro Tools



(a) Separation cell (b) Micro Tools holding chip

Fig. 4 Separation Cell for MT Injection (Top View)

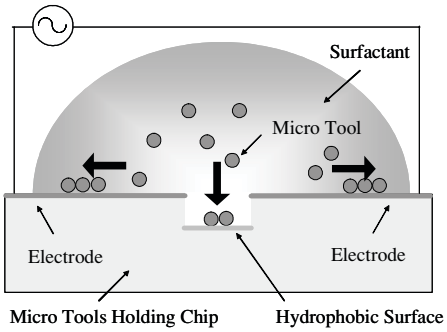


Fig. 5 Alignment of Micro Tools by Negative DEP

Figure 7 shows a MT holding chip made of glass. As a micro tool, we used a polystyrene micro bead (nominal diameter: 10 μm, index of refraction: 1.59 at 589 nm) suspended in the 17% detergent. Polymer density is 1.05 g/cm^3 and suspension density is 1.0 to 1.05 g/cm^3. We applied square wave voltage from -10V and 10V at 1 MHz between the electrodes. Then, we succeeded in alignment of micro beads in the micro groove as shown in Fig. 8. Permittivity of the polystyrene is 2.5 to 2.7 and that of the water at room temperature is 80. In the experiment, the MTs are moved by the negative dielectrophoretic force as illustrated in Fig. 5. The MTs gathered in the micro groove are aligned autonomously by the liquid bridge force of the surface as shown in Fig. 9. The liquid evaporates by heating and the MTs are fixed firmly at the bottom of the micro groove with the water-soluble layer. Then, the MT holding chip is set in the separation cell.

After filling the water, we observed the pinpoint injection of the micro tools at the desired location as shown in Fig. 10. The MTs sank in about 20 to 30 seconds after immersion and the image was defocused as shown in Fig. 10 (b). Then, it was easy to manipulate the polystyrene beads by the laser scanning micromanipulator. The target microbe (yeast) in the separation chamber was manipulated successfully by the laser trapped two polystyrene beads as shown in Fig. 15. Interference of the laser trapped micro tools and the yeast was not significant.

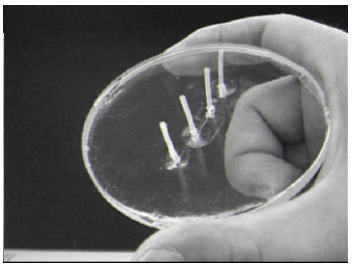
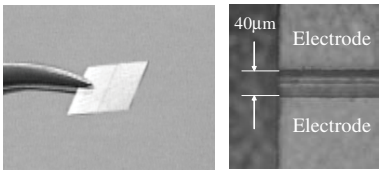
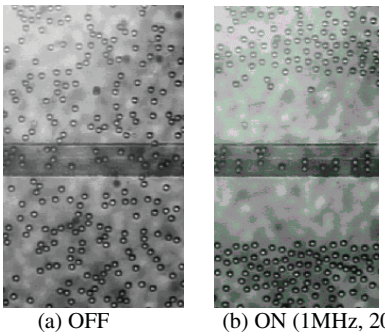


Fig. 6 Separation cell made by the PDMS



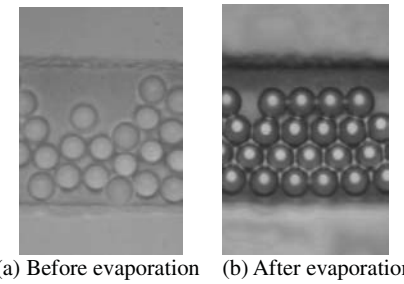
(a) Holding chip (b) Edge of holding chip

Fig. 7 Photo of Micro Tools Holding Chip



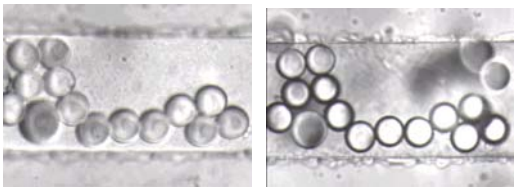
(a) OFF (b) ON (1MHz, 20Vpp)

Fig. 8 Alignment of MTs by Dielectrophoresis



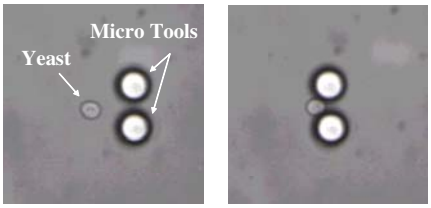
(a) Before evaporation (b) After evaporation

Fig. 9 Alignment of Micro Beads in the Micro Groove



(a) Before immersion (b) After immersion

Fig. 10 Injected MTs (Released calmly in the liquid)



(a) Before pushing (b) Pushing a yeast

Fig. 11 Laser Trapped MTs pushing a Yeast (ϕ 6μm)

CONCLUSIONS

Here we proposed a pinpoint injection method of micro tools at the desired location in the micro chamber, which is filled with liquid. We used the micro tool holding chip and aligned the micro tools in its micro groove autonomously by the dielectrophoresis and liquid bridge force of the hydrophobic surface. The micro tools were injected in the micro chamber, and were manipulated successfully by the laser scanning micromanipulator to separate the target microbe. The proposed system can be applied to the lab-on-a-chip for microbe separation, DNA and protein analysis, and other biological and chemical activities in the micro space.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Melamed, M. R., Lindmo, T., and Mendelsohn, M. L., Flow Cytometry and Sorting, second edition, Wiley-Liss, New York, USA, 1991

[2] Arai, F., Ogawa, M., Fukuda, T., Horio, K., Sone, T., Itoigawa, K., and Maeda, A., High Speed Random Separation of Microobject in Microchip by Laser Manipulator and Dielectrophoresis, Proceedings of IEEE MEMS 2000, Miyazaki, Japan, 2000, p.727-732.

[3] Arai, F., Ichikawa, A., Ogawa, M., Fukuda, T., Horio, K., and Itoigawa, High Speed Separation System of Randomly Suspended Single Living Cells by Laser Trap and Dielectrophoresis, Electrophoresis 2001, 22 No. 2, 2001, pp.283-288

[4] Arai, F., Ichikawa, A., Fukuda, T., Horio, K., Itoigawa, K., Stagnation Point Control by Pressure Balancing in Microchannel for High Speed & High Purity Separation of Microobject, Proc. of IEEE/RSJ Int. Conf. Intelligent Robots and Systems (IROS), 2001, pp.1343-1348

[5] Arai, F., Ogawa, M., Fukuda, T., Indirect Manipulation and Bilateral Control of the Microbe by the Laser Manipulated Microtools, Proc. of IEEE/RSJ Int. Conf. Intelligent Robots and Systems (IROS), VOL. 1, 2000, pp.665-670.

[6] H. Liang, et al., Wavelength Dependence of Cell Cloning Efficiency after Optical Trapping, Biophysical Journal, Vol.70, 1996, pp. 1529-1533

[7] K. Hosokawa and R. Maeda, A pneumatically-actuated three-way microvalve fabricated with polydimethylsiloxane using the membrane transfer technique, J. Micromech. Microeng., 10, 2000, pp.415-420